2014 Harry M. Zweig Memorial Fund for Equine Research Summary Report

The 2014 Annual Report covering the period of January 1, 2014 through December 31, 2014 is enclosed.

For this reporting period, The Harry M. Zweig Memorial Fund for Equine Research Committee granted approval of 6 of 12 submitted projects. Five were new studies, and two were revised. Three continuation awards were also approved. The total amount allocated for 2014 awards was $337,290. Copies of the investigators’ reports are provided.

In honor of the 35th Zweig Fund Anniversary, Cornell hosted a series of research presentations by Cornell faculty at the Cornell Ruffian Equine Specialists, A Cornell University Affiliate Center for Equine Sports Medicine & Critical Care, in Elmont, New York, followed by a tour of the facility and interaction between faculty, staff and Zweig Committee members to learn about research at the College and the Ruffian center. The event took place on Wednesday, November 12, 2014.

2014 Harry M. Zweig Memorial Fund for Equine Research Awards

<table>
<thead>
<tr>
<th>CONTINUATION AWARDS</th>
<th>AWARD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Douglas Antczak</td>
<td>T-Cell Mediated Immunity and Vaccine Development in Horses (2nd year award)</td>
</tr>
<tr>
<td>Thomas Divers</td>
<td>Etiology and Prevention of Equine Serum Hepatitis (Theiler’s Disease) (2nd year award)</td>
</tr>
<tr>
<td>Bettina Wagner</td>
<td>Innate Immune Mechanisms and T-cell Responses to Equine Herpesvirus Type-1 in Latently Infected and Naïve Horses (2nd year award)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>NEW AWARDS</th>
<th>AWARD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dorothy Ainsworth</td>
<td>Fine Mapping of candidate Genes Contributing to Equine Left Recurrent Laryngeal Neuropathy (RLN) Phase II (1 year award)</td>
</tr>
<tr>
<td>Lisa Fortier</td>
<td>Cellular Biomarkers of Early Cartilage Injury Measured with Multiphoton Imaging (1 year award)</td>
</tr>
</tbody>
</table>
Robert Gilbert  Effect of Early Pregnancy on Function of the Equine Corpus Luteum (1 year award)  $49,350

Alan Nixon  Evaluation of Lubricin as New Biotherapeutic for Equine Joint Disease (2 year award)  $81,339

Rolfe Radcliffe  En Bloc Removal of Intravascular Thrombi via an Extracorporeal Bypass Circuit in experimentally Induced Jugular Thrombosis in Horses (1 year award)  $10,000

Bettina Wagner  A Novel Strategy to Boost Antibody Production to EHV-1 in Neonates (2 year award)  $82,945

Interim & Completed 2013 Awards

Dr. Douglas Antczak’s project entitled “T-cell Mediated Immunity and Vaccine Development in Horses.” A final report will included in next year’s report.

Dr. Norm Ducharme’s project entitled “An Exploratory Study into the Practical Application of a Regenerative Medicine Approach to Reconstruction of the Equine Upper Airway.” Dr. Ducharme received an additional no cost extension through June 30, 2015. A Progress report is included herein, and a final report will be included in next year’s report.

Dr. Lisa Fortier’s project entitled “Identification of the Optimal Biologic to Enhance Endogenous Stem Cell Recruitment and Homing for Facilitated Musculoskeletal Tissue Regeneration.” Dr. Fortier received a no cost extension through June 30, 2014. A Final report is included herein.

Dr. Linda Mittel’s renewal project entitled “Detection of Spirochetes, Rickettsia, and other Bacteria and Parasitic Protozoa (often vector born) that Cause Fevers of Unknown Origin in Horses and in Horse-Associated Ticks in the Northeast, Mid-Atlantic, and Great Lakes Regions” received a no cost extension through June 30, 2014. A Progress report is included herein, and the final report will be provided next year.

Dr. Tracy Stokol’s project entitled “The Role of Platelets in the Pathogenesis of Equid Herpes Virus Type-1 Infection.” Dr. Stokol received a no cost extension through December 31, 2014. A Final Report report is included herein.

FURTHER SECURED FUNDING FROM ZWEIG AWARDS IN 2014

The Incentive Program enables the Fund to leverage its investment in Zweig-sponsored research by encouraging Veterinary College faculty to seek either additional or supplementary monies from external sponsors that base their award decisions on a process that involves informed scientific review. The external grant must be closely related to a Zweig project. Eligible sponsors include, but are not limited to, the Grayson Foundation, the NIH, the NSF, and the USDA’s National Research Initiative. Recipients provide an annual report on the use of these funds.
The following external grant awards resulted from Zweig funding:

<table>
<thead>
<tr>
<th>Principal Investigator</th>
<th>External Award</th>
<th>Sponsor</th>
<th>Project Period</th>
<th>Awarded Amount</th>
<th>Incentive Award</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr. Tracy Stokol</td>
<td>Platelet Inhibitors: Potential Antithrombotics for EHV-1</td>
<td>Grayson-Jockey Club</td>
<td>4/1/14-3/31/15</td>
<td>$68,368</td>
<td>$5,000</td>
</tr>
<tr>
<td>Dr. Alan Nixon Heidi Reesink</td>
<td>Lubricin as a Biotherapeutic for Equine Joint Disease</td>
<td>Grayson-Jockey Club</td>
<td>4/1/14-3/31/15</td>
<td>$15,000</td>
<td>$1,500</td>
</tr>
</tbody>
</table>

PUBLICATIONS

Publications resulting from awards from the Harry M. Zweig Memorial Fund for Equine Research during 2014 were:

Genomic analysis establishes correlation between growth and laryngeal neuropathy in Thoroughbreds.  
Boykо AR, Brooks SA, Behan-Braman A, Castelhano M, Corey E, Oliveria KC, Swinburne JE, Todhunter RJ, Zhang Z, Ainsworth DM, Robinson NE  

Ultrasound features of arytenoid chondritis in Thoroughbred horses.  
Garrett KS, Emberton RM, Woodie JB, Cheetham J.  

Computed Tomography-Guided Tissue Engineering of Upper Airway Cartilage  


Regenerative Medicine Approach to Reconstruction of the Equine Upper Airway  

Identification of a previously undescribed divergent virus from the Flaviviridae family in an outbreak of equine serum hepatitis  
Increasing Platelet Concentrations in Leukocyte-Reduced Platelet-Rich Plasma
Boswell, SAG, Schnabel LV, Mohammed, HO, Sundman EA, Minas T, Fortier LA.

The anti-inflammatory and Inflammatory and matrix Restorative Mechanisms of Platelet-Rich Plasma in Osteoarthritis
Sundman EA, Cole BJ, Karas V, Della Valle CJ, Tetreault MW, Fortier LA.


Plasma and synovial fluid concentration of doxycycline following low-dose, low-frequency administration, and resultant inhibition of matrix metalloproteinase-13 from interleukin-stimulated equine synoviocytes
Maher MC, Schnabel LV, Cross JA, Papich MG, Divers TJ, Fortier LA

Identification of cartilage injury using quantitative multiphoton microscopy

Effects of Clopidogrel on Horses with Experimentally Induced Endotoxemia
Antibodies to OspC, OspF and C6 antigens as Indicators for Infection with Borrelia Burgdorferi in Horses. Wagner B, Goodman LB, Rollins A, Freer HS

Genomic Analysis Establishes Correlation between Growth and Laryngeal Neuropathy in Thoroughbreds
Boyko AR, Brooks SA, Behan AL, Castelhano M, Corey E, Oliveira KC, Todhunter RJ, Zhang Z, Ainsworth DM, Robinson NE
http://www.biomedcentral.com/1471-2164/15/259 (2014)

Identification of Cartilage Injury using Quantative Multiphoton Microscopy
Novakofski KD, Williams RM, Fortier, LA, Mohammed HO, Zipfel WR, Bonassar LJ

Equid herpesvirus type 1 activates platelets through tissue factor-triggered thrombin generation.
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4407896/
At the November 15, 2012 Annual meeting, Dr. Jonathan Cheetham was appointed as the first Zweig Research Scientist 2012-2013. His appointment is for the period fiscal year 2014-2015, with was approved at the 2014 annual meeting of the Zweig Committee. Acknowledgement of this prestigious award is included herein.

The Zweig Principal Research Scientist is an exceptional recognition and honor for an early career investigator. It was recognized beyond Cornell and many collaborators and scientists asked Dr. Cheetham about it. This gave him the opportunity to talk about the tremendous impact that the Zweig Memorial support has on equine research at the College of Veterinary Medicine at Cornell University. This award has allowed him to start to pursue a line of investigation and development that will lead to regenerative therapies for equine laryngeal disease.

The ongoing, long-term project will produce a fundamental shift in the way in which horses are treated for Recurrent Laryngeal Neuropathy (RLN), or “Roaring”. This is a significant performance limiting problem in both racehorses and sports horses. In recent years and with support from the Zweig Fund, leveraged into support from the Grayson Jockey Club, Dr. Cheetham’s laboratory has made considerable progress towards understanding the etiology of RLN and making an early diagnosis before terminal fibrotic changes occur. This work is currently under review for publication.

The latest techniques and approaches were presented in abstract from at the Neuroscience meeting in November 2013. Dr. Cheetham’s laboratory’s first papers on each of the approaches were submitted for review December 2014. This work would not have been possible without support from the Harry M Zweig Memorial Fund.
CORNELL CLINICAL FELLOW IN EQUINE HEALTH

At the 2007 Annual meeting, the Harry M. Zweig Committee approved the allocation of funds to help support a Cornell Clinical Fellow in Equine Health. Dr. Sophy Jesty was selected as Cornell’s first Clinical Fellow, followed by Dr. Sarah Pownder, and more recently another individual has been identified as a Clinical Fellow, Dr. Joy Thomlinson and supported in part by Zweig funds, and all have been highly successful. Cornell’s College of Veterinary Medicine’s two-year Clinical Fellows Program is the first in the country to address a growing shortage of academic veterinarians who conduct research on animal diseases and basic biology. The program is designed to help students meet the financial and time demands of qualifying for a position in veterinary academic medicine, which has traditionally required students to complete an M.S. or Ph.D. after they finish their doctorate in veterinary medicine (DVM). The two-year program, available to veterinarians who have completed a three-year residency, offers an annual salary of $65,000 plus benefits and an additional $15,000 per year to fund a research project.


Research project: The effect of macrophage activation phenotype on the regeneration of peripheral nerves.

Dr. Tomlinson recently begun her first year as a Zweig Equine Clinical Fellow, and has provided a brief write up of her experience below for the Zweig Committee. Dr. Tomlinson will be invited to give a presentation at next year’s Zweig poster session and research presentations, scheduled for Wednesday, November 18, 2015 at 3:00pm at the Cornell University’s College of Veterinary Medicine.

“As a clinical fellow, I am starting a project in Dr. Cheetham’s laboratory to investigate ways to improve peripheral nerve regeneration by manipulating the immune response to nerve injury. Our goal is to apply techniques we develop in the lab to improve functional recovery for horses with laryngeal hemiplegia that are treated with nerve graft. During the set-up phase for this project, I have had a chance to spend some time on clinics as well and I am very much looking forward to continuing to stay active in clinical practice while pursuing this research.”

OUTREACH 2014

Patent updates for 2014

During 2014 patent 3080-04-EP - “Cloning & Molecular Characterization of Immunogenic LIG 130 of Leptospira Interrogans” EPC European patent 1565080 was issued to Dr. Chang on January 1, 2015 (including France (1565080), Germany (60345629.4), and the United Kingdom (1565080). College of Veterinary Medicine, Cornell University, Ithaca, NY (2014)

Zweig News Capsules

There were two issues of the Zweig News Capsule published in 2014. Copies of these issues can be found in Appendix (E).

All Zweig News Capsules can be found at the Zweig Website at: http://www.vet.cornell.edu/zweig/

SUMMARY OF EXPENDITURES

The 2014 Summary of Allocations was presented and approved at the Zweig Committee Annual Meeting in November 2013 (Appendix B).

2015 ZWEIG PROGRAM

Nine (9) projects were approved for funding, from a roster of eighteen (18) applications, at the Harry M. Zweig Memorial Fund annual November 2014 meeting. The list of projects funded for 2015 are shown in (Appendix D).
APPENDIX A
Progress & Final Reports Resulting from 2014 Funding

<table>
<thead>
<tr>
<th>Researcher</th>
<th>Project Title and Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr. Ainsworth</td>
<td>Fine Mapping of Candidate Genes contributing to Equine Left Recurrent Laryngeal Neuropathy (RLN) Phase II</td>
</tr>
<tr>
<td>Dr. Antczak</td>
<td>T-Cell Mediated Immunity and Vaccine Development in Horses</td>
</tr>
<tr>
<td>Dr. Divers</td>
<td>Etiology and Prevention of Equine Serum Hepatitis (Theiler’s Disease)</td>
</tr>
<tr>
<td>Dr. Gilbert</td>
<td>Effect of Early Pregnancy on Function of the Equine Corpus Luteum</td>
</tr>
<tr>
<td>Dr. Fortier</td>
<td>Cellular Biomarkers of Early Cartilage Injury Measured with Multiphoton Imaging</td>
</tr>
<tr>
<td>Dr. Nixon</td>
<td>Evaluation of Lubricant as New Biotherapeutic for Equine Joint Disease</td>
</tr>
<tr>
<td>Dr. Radcliffe</td>
<td>En Bloc Removal of Intravascular Thrombi via an Extracorporeal Bypass Circuit in Experimentally Induced Jugular Thrombosis in Horses</td>
</tr>
<tr>
<td>Dr. Wagner</td>
<td>A Novel Strategy to Boost Antibody Production to EHV-1 in Neonates</td>
</tr>
<tr>
<td>Dr. Wagner</td>
<td>Innate Immune Mechanisms and T-Cell Responses to Equine Herpesvirus Type 1 in Latently Infected and Naïve Horses</td>
</tr>
</tbody>
</table>
**Harry M. Zweig Memorial Fund**  
*for Equine Research*

**2014 Progress report**

<table>
<thead>
<tr>
<th>P.I.</th>
<th>Dr. Dorothy Ainsworth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Title:</td>
<td>Fine Mapping of Candidate Genes Contributing to Equine Left Recurrent Laryngeal Neuropathy (RLN)</td>
</tr>
<tr>
<td>Project Period:</td>
<td>1/1/14-12/31/15</td>
</tr>
<tr>
<td>Reporting Period:</td>
<td>1/1/14-12/31/14</td>
</tr>
</tbody>
</table>

Dr. Ainsworth was granted a no cost extension through December 31, 2015. A progress report is attached.
PROJECT TITLE: Fine Mapping of Candidate Genes Contributing to Equine Left Recurrent Laryngeal Neuropathy (RLN): Phase II

PRINCIPAL INVESTIGATOR(S): DM Ainsworth

HYPOTHESIS: In Thoroughbreds, at least one of the genes responsible for the development of recurrent laryngeal neuropathy (RLN) is located on ECA3, in close proximity to, but separate from the equine “size” genes LCORL/NCAPG.

SPECIFIC AIMS: Identify candidate gene(s) associated with RLN in Thoroughbreds by using an across-breed genotype approach. We sought to investigate this disorder in Belgian Draft Horses which reportedly have a 35% prevalence rate of RLN.

We will phenotype and genotype 400 Belgian Horses (200-RLN affected–grade 4; 200 controls-grade 1) using the EqSNP70 Beadchip and our custom-designed array containing 203 SNPs.

We recognize that identification of additional SNPs may need to be added to the custom-designed array (which we have previously designed for the Thoroughbreds), based upon the genotyping results of the Belgian horses using the EqSNP70 chip. We are prepared to do so if necessary.

Results:

To date we have phenotyped and genotyped 277 Belgian horses. The cold and early-onset winter precluded us from obtaining samples from November (2014) until April (2015). However, we have currently resumed our sample collection efforts and our collaborator, Dr. Ed Robinson from MSU, recently delivered 95 samples for our project (obtained at a recent Draft Horse Show). The samples are currently in the CU DNA bank waiting to be processed (DNA isolated). The PI, after speaking recently to members at a NYS Draft Horse Association meeting, has lined up several farm visits in upstate NY in June and July for sample collection. The PI will also be attending a Draft Horse Pull Contest in late May (in PA) to obtain additional Belgian horse samples. In addition, the PI has been obtaining samples from clinical cases that are presenting to E/FAH. Our goal is to phenotype and genotype an additional 200 Belgian horses by October 2015. We aim to complete data analysis by December 2015 and submit an abstract for presentation during the 2016 year. Preparation and submission of an abstract would follow shortly thereafter.

Conclusions and significance: Although we had hoped that the Belgian horse genomic analysis would “break open” the region on ECA3 near LCORL/NCAPG identified in the Thoroughbreds, our approach using Belgian horses will still yield valuable insight into the pathogenesis of RLN. This will be especially true if the ECA1 locus suggested in preliminary data turns out to be located near growth genes for the Belgian Draft horse.

Publications: None at this point.
Harry M. Zweig Memorial Fund  
for Equine Research

2014 Progress Report

<table>
<thead>
<tr>
<th>P.I.:</th>
<th>Dr. Thomas Divers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Title:</td>
<td>Etiology and Prevention of Equine Serum Hepatitis (Theiler’s Disease)</td>
</tr>
<tr>
<td>Project Period:</td>
<td>1/1/13-6/30/15</td>
</tr>
<tr>
<td>Reporting Period</td>
<td>1/1/14-12/31/14</td>
</tr>
</tbody>
</table>

Dr. Divers was granted a no cost extension through June 30, 2015. A progress report is provided (a final report will be included next year).
PROGRESS REPORT

PROJECT TITLE: Etiology and Prevention of Equine Serum Hepatitis (Theiler’s Disease)

PRINCIPAL INVESTIGATOR(S): Thomas Divers with Bud Tennant

Specific Aims: The three specific aims have not changed.

Specific Aim 1. Express clones of EHAV antigen sequences, purify the recombinant viral proteins and develop serologic tests for detection of EHAV antibodies. Expression of viral proteins and the development of immunosorbent antibody tests will be under the direction of Dr. Y.C. Change. The tests for EHAV antibodies are essential for the conduct of the second and third specific aims.

Specific Aim 2. The second specific aim will be to conduct a cross sectional study to assess the extent of exposure to EHAV. The target population will be North American horses. Consortium members will solicit participation of farms across the United States and Canada. Adult horses on the farm will be surveyed serologically to determine the extent of exposure to EHAV and the number of horses that are infected (EHA virus positive) or have been infected (EHAV antibody positive, EHA virus negative). The prevalence of EHAV infection in adult horses will be determined based on the presence of EHA viremia detected by RT-PCR, a procedure that currently is available, and the presence of EHAV antibodies to be detected by immunosorbent assays to be developed (Specific Aim 1).

Specific Aim 3. The third specific aim will be a prospective case control study, the purpose of which will be to determine the frequency of productive EHAV infection in successive new cases of acute hepatitis/Theiler’s disease. The risk of EHAV in Theiler’s disease cases will be compared to age and gender matched controls.

Specific Aim 1 - Studies and Results: Four genes of the Theiler’s disease associated virus (TDAV) were selected, expressed in E. coli, and the expressed protein purified. Western blot testing of the 4 proteins was performed using serum positive for TDAV by PCR and serum from a herd of horses that were infected for TDAV virus and were PCR negative. The expresses NS5A gene was found to provide the most accurate testing. This 30 Kd protein (30 Kd) was tested further by Western blot to establish the most appropriate concentrations of protein for Western blot testing. The NS5A protein will be used as the protein antigen in an ELISA for TDAV antibody detection. The projected completion of this aim is January-February 2014. Development of the ELISA is being performed in the laboratory of DR. Yung-Fu Chang.

Specific Aim 2 - Studies and Results: While the ELISA is being completed, collaborative arrangements are being established by Dr. Bruce Akey, Executive Director of the Cornell University Animal Health Diagnostic Center, to obtain serum samples from collaborating regional diagnostic centers from across North America to determine the prevalence of TDAV infection. Seven North American regional diagnostic laboratories will be recruited from the northeastern, southeastern, mid-western, southwestern and western regions of the United States and from an eastern and a western province of Canada. Serum samples from approximately 100 horses will be provided by each collaborating laboratory by using the residual serum from samples submitted for routine Equine Infectious Anemia testing. The geographic origin of each
horse will be provided and age, gender, and breed will be obtained if possible.

**Specific Aim 3:** We are not aware of additional outbreaks of Theiler’s disease within the past six months. We have received samples from two isolated suspect cases both of which were PCR negative. A letter has been sent to 25 colleges and large private practices throughout the United States and Canada trying to recruit cases and age matched controls. We have continued to monitor the Nevada horses involved in the original outbreak and just recently (September 2013), identified two horses that remain PCR positive for TDAV, two years following antitoxin treatment and the hepatitis outbreak. Drs. Randy Renshaw and Ed Dubovi have performed the PCR testing on these recent samples.

Our 2013 findings further confirms that a low percentage of infected horses can become chronic carriers of the virus and these horses would be a risk for transmitting infection and possibly disease if used for equine serum/plasma production. If we find additional cases of acute hepatitis associated with TDAV infection we believe official testing will be needed for all equine plasma/serum products. Our intention is that any official testing will be performed at the Animal Health Diagnostic Center at Cornell University. There is so far no evidence of horizontal spread in the Nevada herd.

There are no modifications of the original plan. Drs. Tennant, Dr. Van de Walle, and Divers met with virologists at Rockefeller University and Columbia University and have submitted a joint NIH grant application with both groups to support further experimental studies of TDAV. The proposal includes the construction of a full length clone of TDAV, the establishment of its infectivity in vitro in cultured equine hepatocytes, and ultimately the cloned TDAV will be transfected (by direct hypodermic needle injection) into the liver liver of experimental horses. Koch’s postulates for TDAV would be fulfilled if a productive TDAV infection developed following transfection of the cloned TDAV.
## 2014 Progress Report

<table>
<thead>
<tr>
<th>P.I.</th>
<th>Dr. Norm Ducharme</th>
</tr>
</thead>
<tbody>
<tr>
<td>Title:</td>
<td>An Exploratory Study into the Practical Application of a Regenerative Medicine Approach to Reconstruction of the Equine Upper Airway</td>
</tr>
<tr>
<td>Project Period:</td>
<td>1/1/13-6/30/15</td>
</tr>
<tr>
<td>Reporting Period:</td>
<td>1/1/14-12/31/14</td>
</tr>
</tbody>
</table>

Dr. Ducharme was granted a no cost extension through June 30, 2015. A progress report is provided (a final report will be included next year).
Project Title: An exploratory study into the practical application of a regenerative medicine approach to reconstruction of the equine upper airway

Principal Investigator: Norm Ducharme

The research entitled “An exploratory study into the practical application of a regenerative medicine approach to reconstruction of the equine upper airway” aims to determine effective methods for the replacement of laryngeal cartilage using decellularized equine cartilage as treatment of cartilage abnormalities leading to airway obstruction. These regenerative medicine strategies require at first the development of efficient methods for the decellularization of equine cartilages, and subsequently the verification of performance of the scaffold based implants in a clinical model of laryngeal reconstruction. The two specific aims of the study, to be conducted in two distinct and consecutive phases, were to:

1) Develop decellularization methods for the production of mechanically robust, geometrically accurate scaffolds from the equine cartilages while retaining the tissue specific ultrastructure and functional composition of the source tissues
2) Determine the ability of these acellular scaffold materials with and without bone marrow derived cells, to integrate and function as inductive templates for the reconstruction of the equine upper airway following partial resection of the epiglottis and arytenoid in vivo.

The first 11 months of the project focused on Aim 1, identifying the most effective protocol for decellularization of equine laryngeal cartilages.

Phase 1
Complications encountered and solved

1. Several issues affected the time to effectively start the Phase 1 and its duration. The delay was associated with the difficulty in collecting laryngeal specimen at Cornell University. This was resolved by importing fresh frozen equine larynges from abattoir in Canada. The delay was associated with the acquisition of proper import permits.
2. Once the best decellularization method was identified and the production of equine laryngeal cartilage scaffolds started, together with the first implant surgery, a technical issue with the equipment used in the final step of scaffold production (lyophilization) affected the quality of the scaffolds leading to a forced pause in the study progress, waiting for the problem to be solved and new implants to be prepared. The recurrence of the above mentioned technical impairment suggested a new approach for the scaffold use that could avoid the final lyophilization step. The implants sets were kept in their decellularized but hydrated status and shipped overnight soon before surgery.

Larynges (n=50) were harvested in the early post-mortem period from horses underwent euthanasia at Cornell University for reason unrelated to the study or from horses slaughtered at the Montreal abattoir. After collection, the larynges were frozen, stored at -20°C, and shipped overnight on dry ice to the McGowan Institute laboratories, where storage was maintained at -80°C. The Co-PI (Brown) processed an initial cluster of laryngeal samples according to 3 distinct decellularization methods, as described in the proposal. Samples resulting from each method underwent qualitative and quantitative assessment of decellularization efficacy; macro-, micro-, and ultrastructure evaluation to determine maintenance of 3-dimensional geometry, and biomechanical evaluation of the decellularized scaffold. Quantitative measurements resulted from the scaffolds obtained from each decellularization method were compared to the characteristics of the native laryngeal cartilages. The method that produced cartilage scaffolds with the highest rate of decellularization associated at mechanical and structural characteristics closest to the native
ones resulted to be Method #1. Method 1 consists of an overnight water wash followed by incubation in a solution of Triton-X100 to achieve decellularization. The scaffold materials are then further washed and disinfected using peracetic acid. Method 1 was further refined to include 72h in Triton-X100 as it produced better decellularization than 48h. Based on these results, the remaining laryngeal samples were processed according to the above mention method to produce decellularized scaffold implants to be used in the in vivo phase. Hydrated larynges were determined to be more conducive to surgical implantation as the sutures could be placed easily without the impression of stress fracture associated with needle holes from the suture.

Results: The decellularization protocol used for preparation of the implants was optimized by comparing three separate decellularization procedures. The results were evaluated histologically, and biochemically to assess removal of cellular content as well as maintenance of tissue architecture and content. The optimized procedure was then used to create materials for implantation.

Phase 2

Horses donated to Cornell University were examined to determine the native laryngeal function at rest to ensure normal structural and anatomical status and full abduction during exercise. Indeed, horses showing laryngeal cartilages abnormality, arytenoid collapse (laryngeal grade B and C) or intermittent dorsal displacement of the soft palate during exercise were excluded from the project. Horses not experiencing upper airway obstruction during exercise were included in the study and started a daily training on treadmill. When the horses reached a level of fitness adequate to sustain a maximal exercise, determination of maximal heart rate during strenuous exercise was performed according to the standard protocol described in the proposal. Heart rate values recorded during the test was regressed on treadmill speed, and the speeds predicted to produce 50, 80, 90 and 100% $HR_{max}$ were determined for each horse. Laryngeal transcutaneous ultrasound was performed in each horse to evaluate arytenoid cartilage structure and thickness, and exclude the presence of underlying arytenoid condritis or calcification of the cartilage. Prior to surgery each horse underwent a treadmill test to determine the native biomechanics of the larynx through a 4 intervals exercise at increasing speed to produce respectively 50, 80, 90 and 100% $HR_{max}$, while recording videoendoscopy and airways pressure.

Few hours before surgery each horse was sedated and bone marrow samples were collected through aspiration from 2 or 3 distinct sternebrae. The bone marrow aspirate was diluted in phosphate buffered solution (PBS) and subjected to Ficoll density gradient centrifugation to extract the white blood cells fraction, afterwards rinsed and diluted in PBS with antibiotics. Scaffolds were seeded with bone marrow derived cells by immersion of the sample in the enriched final cell solution for at least 30 minutes prior to implantation. Horses were grouped in couple and assigned to a specific timeline of 1, 2, 3 or 4 months. The combination of cells seeded/unseeded implants was randomly assigned for each couple of horses, to obtain at each timeline one cartilage with unseeded implant and one implanted with scaffold seeded in cells.

The first definitive set of scaffolds (epiglottis, right and left side arytenoids) obtained from the Phase 1 of the project was ready for implantation on December 2013. The first horse underwent surgery on December 9th 2013. After induction, tracheostomy was performed to allow inhalator general anesthesia while performing surgery through laryngotomy. A custom-made tool has been specifically designed for the project to allow a consistent approach to native cartilage explant and scaffold implantation, able to create cartilage biopsy specimen of 1cm diameter through a laryngotomy approach. Each scaffold implanted was secured in place with non-absorbable suture and then covered with decellularized sheets of porcine bladder mucosa to reduce traumatism and contamination of the implants during normal physiologic laryngeal movement. The explanted native cartilages were stored in fixative solution (formaldehyde 10%) for following tests of their biomechanical content and strength, serving as control for the explants scaffold at the end of the in-vivo phase. Also a portion of scaffold seeded in cells was collected and stored in fixative to perform evaluation on electron microscopy of the infiltrative capacity of the cells into the scaffold.

Each horse was examined daily for general condition and evidence of aspiration. Resting endoscopic exam and laryngeal ultrasound were done 24hrs after surgery, then at weekly intervals for the first month and monthly thereafter to assess the integrity of the repair. Tracheostomy was maintained for at least 24hrs or till laryngeal patency was confirmed endoscopically.

In the period December 2013-April 2014, 8 horses underwent surgery for this project, 2 horses for each timeline (1, 2, 3 and 4 months). Bone marrow aspiration was performed in all horses without complications. All horses recovered
uneventfully from general anesthesia, all but 1 had tracheostomy tube removed within the 1st week post-surgery. One horse, previously assigned at the month timeline, experienced the development of a peri-laryngeal abscess causing airway obstruction that required the maintenance of the tracheostomy for 3 weeks.

The first three horses to have surgery lost the epiglottis implant within the first 2 weeks due to suture cutting through the scaffold or through the native cartilage, with subsequent entrapment of the remaining epiglottis. The complications recorded with the epiglottis implant suggested proceeding with a different approach for the epiglottis implantation, as placement of the implant between the native cartilage and the ary-epiglottis fold.

Two arytenoid implants were extruded (12.5%) but did so without complications. Three horses, two at 1 month and one horse at 2 months, underwent euthanasia as per protocol; the whole larynx was explanted, stored in fixative solution and shipped to the Co-PI for the biomechanical and biochemical testing, and histologic analysis. All live animals studies were concluded on October 12, 2014.

Results: During the course of the phase 2 study it was found that the use of freeze dried implants presented a challenge due to their stiffness and inability to be shaped to the size of the defect to be repaired. Thus, the protocol was adjusted to allow the use of fully hydrated implants. Hydrated implants were observed to perform better both in terms of handling by the surgeon as well as upon examination endoscopically. Thus, the majority of implantations were performed using hydrated implants. The remodeling of the implants was assessed endoscopically at multiple time points. Results suggest progressive remodeling of the implants and integration within airway tissues over time. Histologic analysis was performed to confirm the gross morphologic assessment. Histologic analysis demonstrates progressive degradation of the implant and replacement with host tissues over time. The tissue formed consisted of both collagenous tissues and newly formed cartilage beginning at the periphery of the implant and increasing with time of implantation. These results suggest successful generation of implants for reconstruction of the equine upper airway as well as appropriate response to the implants and development of new tissues over time. Further staining and analysis is under study and the blind-control key has not been broken yet to allow for accurate evaluation. Statistical analysis will follow.
**Harry M. Zweig Memorial Fund**  
for Equine Research

**2014 Final Report**

<table>
<thead>
<tr>
<th>P.I.</th>
<th>Dr. Lisa Fortier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Title:</td>
<td>Cellular Biomarkers of Early Cartilage Injury Measured with Multiphoton Imaging</td>
</tr>
<tr>
<td>Project Period:</td>
<td>1/1/13-6/30/15</td>
</tr>
<tr>
<td>Reporting Period</td>
<td>1/1/14-12/31/14</td>
</tr>
</tbody>
</table>

Dr. Fortier received a no cost extension through June 30, 2015. A Final report is included herein.
Project Title: Cellular Biomarkers of Early Cartilage Injury Measured with Multiphoton Imaging
Principal Investigator(s): Lisa Fortier

This project is complete and has resulted in the following three manuscripts.


Any of these manuscripts are available upon request by emailing Dr. Lisa Fortier (laf4@cornell.edu). In addition, these projects served as the central theme for the Ph.D. studies of Dr. Kira Novakofski whom went on to Albany Medical School after completing her Ph.D. Kira secured stipend funding for this project from the CTSC program which is a highly competitive funding program focused on research with promising translational application.

This work continues in my laboratory with Dr. Michelle Delco whom is a boarded equine surgeon seeking a Ph.D. We are seeking funding for the project in multiple avenues having not been successful in the 2015 Zweig program. The results of these studies will have a significant impact on how to diagnose subtle cartilage injuries before the onset of osteoarthritis. The goal is to prevent the development of osteoarthritis.
### Harry M. Zweig Memorial Fund for Equine Research

#### 2014 Final Report

<table>
<thead>
<tr>
<th>P.I.:</th>
<th>Dr. Lisa Fortier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Title:</td>
<td>Identification of the Optimal biologic to Enhance Endogenous stem cell recruitment and Homing for Facilitated Musculoskeletal Tissue Regeneration.</td>
</tr>
<tr>
<td>Project Period:</td>
<td>1/1/12-12/31/14</td>
</tr>
<tr>
<td>Reporting Period</td>
<td>1/1/14-12/31/14</td>
</tr>
</tbody>
</table>
PROJECT TITLE: Identification of the Optimal biologic to Enhance Endogenous stem cell

“Identification of the optimal biologic to enhance endogenous stem cell recruitment and homing for facilitated musculoskeletal tissue regeneration.”

This project is complete and a manuscript is in review at the Journal of Orthopedic Research. The title page and abstract follow. A full copy of the manuscript is available upon request by emailing Dr. Lisa Fortier at laf4@cornell.edu. This project also served as the primary project to award a Master’s Degree to Cornell University DVM student Ms. Hannah Holmes. The results of this project will have an immediate effect on clinical practice. We show that by use of bone marrow concentrate, you can optimize recruitment of stem cells to a site of injury.
**Harry M. Zweig Memorial Fund for Equine Research**

**2014 Progress Report**

<table>
<thead>
<tr>
<th>P.I.</th>
<th>Dr. Robert Gilbert</th>
</tr>
</thead>
<tbody>
<tr>
<td>Title:</td>
<td>Effect of Early Pregnancy on Function of the Equine Corpus Luteum</td>
</tr>
<tr>
<td>Project Period:</td>
<td>1/1/14-6/30/15</td>
</tr>
<tr>
<td>Reporting Period</td>
<td>1/1/14-12/31/14</td>
</tr>
</tbody>
</table>

Dr. Gilbert received a no cost extension through June 30, 2015. A progress report is provided.
2014 HARRY M. ZWEIG MEMORIAL FUND FOR EQUINE RESEARCH PROGRAM PROGRESS REPORT

PROJECT TITLE: The Effect of Early Pregnancy on the Equine Corpus Luteum

Principal Investigator(s): Robert O. Gilbert

This project is funded by the Harry M. Zweig Memorial Fund. Its aim is to confirm our earlier observation that circulating progesterone concentrations are higher 5 days after ovulation in pregnant than in non-pregnant horses, and to investigate the mechanism for this phenomenon.

To date we have collected blood samples from approximately 100 cycles (pregnant, non-pregnant and non-bred) to confirm increased progesterone concentrations in pregnant mares, thus completing Aim 1 and Aim 2.

Aim 1.
Embryo recovery and culture to provide embryo-conditioned medium to examine the direct effect of embryo secretions on luteal secretion of progesterone in vitro

Aim 2.
The rest of Aim 2 (luteal biopsy and culture) and luteal biopsy for study of expression of genes of enzymes important in steroidogenesis is underway. Primers for quantitative PCR are in hand.
Harry M. Zweig Memorial Fund
for Equine Research

2014 Progress Report

<table>
<thead>
<tr>
<th>P.I.:</th>
<th>Dr. Linda Mittel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Title:</td>
<td>Detection of Spirochetes, Rickettsia, and other Bacteria and Parasitic Protozoa (often vector borne) that cause Fevers of Unknown Origin in Horses in the Northeast, Mid-Atlantic, and Great Lakes areas of the U.S.</td>
</tr>
<tr>
<td>Project Period:</td>
<td>1/1/12-6/30/14</td>
</tr>
<tr>
<td>Reporting Period</td>
<td>1/1/14-6/30/14</td>
</tr>
</tbody>
</table>

Dr. Mittel was awarded an additional no cost extension through June 30, 2014. A progress report is provided (a final report will be included next year).
Detection of Spirochetes, Rickettsia, and other Bacteria and Parasitic Protozoa (often vector borne) that cause Fevers of Unknown Origin in Horses in the Northeast, Mid-Atlantic, and Great Lakes areas of the U.S.

Principal Investigator(s): Linda Mittel with Amy Glaser

Aim: Identify the presence of potential tick-transmitted (Anaplasma sp., Babesia sp, Borrelia sp, Ehrlichia sp., Rickettsia sp. or other non-respiratory bacterial infections(Leptospira, Bartonella sp., Neorickettsia sp. ) in horses with FUO and controls from the same premise, in clinical practices In the North-East, Mid-Atlantic, and Great Lakes areas of the United States.

We have received 132 samples from all the areas of study including 81 fever cases, 51 controls and 16 ticks. Although we were hoping to receive multiple samples from the same practices, some practices sent a lower number than initially was suggested. We had been contacted by other practices that were interested in the study and due to lower number of samples received from the targeted practices; we accepted these additional samples from similar geographical areas. We also received samples without controls. We contacted the DVMs when possible and had a control animal sample sent even if not taken the same day as the initial sample.

We identified 11 Anaplasma phagocytophilum positive samples and 2 Neorickettsia risticii out of 132 samples.

Sequence analysis of the 16 S portion of the library confirmed the presence of Anaplasma phagocytophilum in all clinical samples in which it had been identified by direct PCR. In addition, sequences mapping to additional Anaplasma and Ehrlichia species were identified in the same samples with frequencies of between 2 and 10% of the number of reads mapping to A. phagocytophilum. This suggests that many of these clinical cases were potentially caused by multiple infections. The identification of multiple Anaplasma/Ehrlichia species in these samples is being confirmed by analyzing for other independently amplified regions from these bacteria present in sequencing library. No Anaplasma/Ehrlichial sequences were identified in any of the control samples. In addition, several potential bacterial pathogens were identified from clinical cases that were not identified in control cases. Several of these have been identified as potential pathogens in hospital settings in humans.

2) Determine the correlation between the presence of identified agents with fever in the study subjects. We have not completed the complete study to determine this correlation.

3) Identify ticks and determine the prevalence of tick-associated pathogens in ticks in the same environment as the samples and control horses and determine the association between tick pathogen load and the likelihood of causing fever in horses from the participating locations.

We received 16 ticks. One tick was positive for Borrelia burgdorferi and 5 ticks positive for Anaplasma.

The collection of clinical samples for this project and testing for Borrelia burgdorferi and Anaplasma phagocytophilum in clinical samples is completed. Because of concerns regarding the potential lack of sensitivity for direct detection of Bartonella species peripheral blood samples,
samples from all EDTA and SPS sterile blood culture tubes submitted for cases and controls were cultured for 4 weeks in two bacterial growth medias used to propagate Bartonella sp. Samples were obtained from the cultures weekly for 4 weeks and analyzed for the presence of Bartonella species. No amplification products matching Bartonella species were identified, but sequences with both common and unique melt curve characteristics were identified in multiple samples

Analysis of the sequence information obtained from the collected samples for the detection additional pathogens listed above is in progress. The unique nature of the approach for pathogen detection required the de-novo development of analysis routines. However, once these analysis pipelines are established, subsequent studies of this type will result in much faster results.

*Horses with FUOs that we sampled appear to have an additional agents causing fever due to the few number of positive Anaplasma and Potomac horse fevers found.* Additional unique products identified following targeted amplification suggest that additional agents are present in the sample set studied.

*Publications and other grant submissions*

Pending completion of the proposed study.
### 2014 Annual Report

<table>
<thead>
<tr>
<th>P.I.:</th>
<th>Dr. Alan Nixon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Title:</td>
<td>Evaluation of Lubricin as a New Biotherapeutic for Equine Joint Disease</td>
</tr>
<tr>
<td>Project Period:</td>
<td>1/1/13-12/31/14</td>
</tr>
<tr>
<td>Reporting Period:</td>
<td>1/1/14-12/31/14</td>
</tr>
</tbody>
</table>
PROJECT TITLE: Evaluation of Lubricin as a New Biotherapeutic for Equine Disease

PRINCIPAL INVESTIGATOR(S): Alan J Nixon

Aim 1. Determine lubricin levels and expression in normal joints and those with synovitis and OA.

Aim 2. Investigate how mechanical properties of the lubricin boundary layer emerge from its nanoscale organization and galectin crosslinking.

Aim 3. Delineate the importance of specific molecular domains in the functional assembly of the lubricin boundary layer.

Aim 4. Determine how cells adhere and migrate along or through the lubricin boundary layer.

Aim 1. Determine lubricin levels and expression in normal joints and those with synovitis and OA.

Lubricin, a mucinous glycoprotein encoded by the proteoglycan4 (PRG4) gene, functions as a boundary lubricant and chondroprotective agent in synovial joints. Mounting evidence suggests that lubricin delays the progression of osteoarthritis (OA), but data indicating how lubricin is altered in the equine athlete is lacking. Our objective was to assess changes in PRG4 gene expression and lubricin synovial fluid concentrations in both naturally occurring and experimental models of equine OA. Lubricin synovial fluid concentrations and PRG4 gene expression were analyzed in horses undergoing carpal osteochondral fragmentation (n=8) and in horses with naturally occurring OA or normal carpi (n=58). Thirty-six horses with naturally occurring carpal OA and twenty-two horses with clinically normal carpi were included. PRG4 gene expression was quantified from synovial membrane and cartilage using qRT-PCR. Synovial fluid lubricin was quantified using a sandwich ELISA. Lubricin concentrations increased in synovial fluid following OA induction (p=0.0003), peaking at 21 days post-operatively (Fig 1).

Lubricin concentrations were significantly elevated in OA vs. normal joints in horses with naturally occurring OA (Fig 2, p=0.0157). Synovial membrane PRG4 gene expression increased in naturally occurring OA (p=0.0025); whereas cartilage PRG4 transcription levels decreased (p=0.0248). Contrary to findings in human and mouse, our results indicate that equine synovial fluid lubricin concentrations increase in response to OA. These data suggest that the role of lubricin in equine OA is more complex than simple down-regulation of lubricin expression, and may involve post-translational modifications, such as glycosylation.

Figure 1. (A) Synovial fluid lubricin concentrations sampled immediately prior to carpal arthroscopy (Day 0) and at weekly intervals after carpal osteochondral fragment induction (OA, n=8) vs. sham-operated controls (Control, n=8). (B) PRG4 transcription levels in synovial membrane from control (n=8) and OA (n=8) joints at day 70 after carpal osteochondral fragment induction. There were no significant differences in PRG4 levels between groups (p<0.05). Data are presented as mean ± s.e.

Figure 2. Synovial fluid lubricin concentrations from naturally occurring OA cases. Lubricin concentrations in OA joints were significantly elevated as compared to normal joints. Differing letters indicate significant difference at p<0.05. Data are presented as mean ± s.e.

Lubricin concentrations were significantly elevated in OA vs. normal joints in horses with naturally occurring OA (Fig 2, p=0.0157). Synovial membrane PRG4 gene expression increased in naturally occurring OA (p=0.0025); whereas cartilage PRG4 transcription levels decreased (p=0.0248). Contrary to findings in human and mouse, our results indicate that equine synovial fluid lubricin concentrations increase in response to OA. These data suggest that the role of lubricin in equine OA is more complex than simple down-regulation of lubricin expression, and may involve post-translational modifications, such as glycosylation.
Aim 2. Investigate how mechanical properties of the lubricin boundary layer emerge from its nanoscale organization and galectin crosslinking.

The ability of lubricin to decrease friction is attributed to its central mucin-rich domain, which is extensively modified via post-translational glycosylation. Galectins, a family of carbohydrate binding proteins, have been detected in altered concentrations in rheumatoid arthritis (RA) and osteoarthritis (OA). Elevations in galectin-3 concentrations in sera and synovial fluid have been associated with RA and OA[1], and elevated galectin-3:galectin-1 ratios have been detected in patients with juvenile idiopathic arthritis[2,3]. However, it remains to be demonstrated whether alterations in galectin expression precede the development of arthritis, and the functions of synovial fluid galectins are incompletely understood.

Galectin-3 was demonstrated to bind lubricin with high-affinity, with a measured dissociation constant of 12nM (Fig 1). Asialofetuin, a protein known to bind galectins with high-affinity, was calculated to bind galectin-3 with a dissociation constant of 1.5nM. Conversely, galectin-1 did not demonstrate lubricin-specific binding, though it did bind to asialofetuin with a dissociation constant of 51nM. Following removal of terminal sialic acid residues on lubricin with sialidase, the number of binding sites for both galectin-1 and galectin-3 increased, as evidenced by increased absorbance values. However, the dissociation constants remained similar before and after sialidase treatment, indicating that removal of sialic acid increased the availability of galectin binding sites but did not alter the affinity of lubricin-galectin binding. In the presence of lubricin, galectin-3 decreased friction coefficients by 13% (p=0.0422), whereas galectin-3 did not significantly reduce friction for cartilage explants in which lubricin had been extracted with 1.5M NaCl (Fig 3). Here, we have demonstrated the first evidence that galectins can bind to lubricin and that high affinity lubricin-galectin-3 binding enhances cartilage boundary lubrication. Together, our results suggest a novel mechanism whereby galectin-3 may crosslink lubricin on the surface of articular cartilage, thereby facilitating boundary mode lubrication and protecting against cartilage damage and subsequent arthritis. This raises the possibility that elevated galectin-3 expression in synovial joints may be a protective mechanism to prevent cartilage degradation following trauma and joint inflammation as opposed to a prequel to arthritis. Future investigation into how lubricin glycosylation is altered in arthritis and how these alterations impact galectin-3 binding and cartilage lubrication will be critical in fully understanding the role of galectins in cartilage biomechanics.

![Figure 1](image1.png)

**Figure 1.** (A) Equilibrium galectin-1 and (B) equine galectin-3 binding to starch lectin (ASL). Galectin-3 demonstrated higher affinity binding to ASL (KD = 15 nM) than galectin-1 (KD = 49 nM). Binding was inhibited in a dose-dependent fashion in the presence of β-lactose. (C) Equine galectin-1 and (D) equine galectin-3 binding to equine lectin (KD = 120 nM), demonstrating inhibition in the presence of β-lactose.

**Figure 3.** Equilibrium friction coefficients for neonatal bovine cartilage explants incubated in phosphate buffered saline (PBS) or 50 μg/mL of rhGalectin-3. Explants were tested in the presence of endogenous lubricin or after lubricin was extracted with a 20-minute incubation in 1.5M NaCl, followed by re-equilibration for 1 hr in PBS. Data are presented as mean ± s.d. with n=4.

Aim 3. Delineate the importance of specific molecular domains in the functional assembly of the lubricin boundary layer.

We recently obtained a codon-optimized construct for human PRG4 and are currently working on expressing this construct in mammalian cell lines.

Aim 4. Determine how cells adhere and migrate along or through the lubricin boundary layer.

Preliminary work has focused on
<table>
<thead>
<tr>
<th>P.I.:</th>
<th>Dr. Rolfe Radcliffe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Title:</td>
<td>En Bloc Removal of Intravascular Thrombi via an extracorporeal Bypass Circuit in Experimentally Induced Jugular Thrombosis in Horses</td>
</tr>
<tr>
<td>Project Period:</td>
<td>1/1/14-12/31/14</td>
</tr>
<tr>
<td>Reporting Period</td>
<td>1/1/14-14-12/31/14</td>
</tr>
</tbody>
</table>
PROJECT TITLE: En Bloc Removal of Intravascular Thrombi via an extracorporeal Bypass Circuit in Experimentally Induced Jugular Thrombosis in Horses

PRINCIPAL INVESTIGATOR(S): Rolfe Radcliffe

The investigators were funded via this Zweig Grant for Part 1 of a two part project and completed phase one of the project during March – May of 2014. Eight horses were used for the first part of the project instead of the six proposed, as we had some difficulty obtaining complete jugular thrombosis on two large Thoroughbred horses. Therefore, we added two horses to obtain adequate numbers for comparison with the planned treatment group next year. Later in the study period, this year we returned to treat the two most difficult horses, and obtained partial and complete thrombosis in these animals. Of the eight horses in Part 1 of the study, the investigators were successful in inducing partial or complete thrombosis in all eight animals.

Our primary objective for Part 1 of the study was to create and validate an experimental model of jugular thrombosis in horses. Specifically we wanted to answer the following questions during Part 1 of this study:

1) Is induction of jugular thrombosis in horses possible with the use of 10% ferric chloride induced endothelial damage via direct catheter injection combined with temporary venous stasis?
2) If 10% ferric chloride is not sufficient in all horses for induction of jugular thrombosis will a higher concentration improve the thrombosis model?
3) What duration of venous stasis following ferric chloride injection is required to induce complete jugular thrombosis in horses?
4) What percentage of the vessel lumen becomes thrombosed after ferric chloride injection, and how many injections are necessary?
5) If jugular thrombosis is successful, how long does jugular thrombosis remain, and what changes occur during the 30 day experimental period?
6) What complications if any result from the direct injection of ferric chloride into the jugular vein of horses?
7) Lastly, is the Angio-Vac Cannula System feasible as a treatment for jugular thrombosis in horses?

Accomplishments towards above objectives:
1) Venous thrombosis via direct injection of 10 to 20% ferric chloride into the jugular vein of horses in concert with temporary venous occlusion consistently induced partial to complete jugular thrombosis. Successful thrombosis was achieved in all eight horses, although individual horse variability was present.
2) The 10% concentration of ferric chloride was sufficient in four horses to achieve complete to near-complete jugular thrombosis (80-100%), while the two most difficult horses required repeated injections of 10% and then 20% ferric chloride for partial (75%) and complete vessel occlusion. The last two horses in our study received 20% ferric chloride only resulting in 90% and 100% thrombosis.
3) The investigators varied the length of venous stasis (applied via proximal and distal tourniquet application), from 10 to 120 minutes following ferric chloride injection, depending upon the initial experimental response in horses. Although the first two horses attained complete venous occlusion with a few (2-3) 10 to 20 minute periods of blood stasis, most subsequent horses required multiple (3-9) 30 to 120 minute periods depending upon initial injection response.

4) Of the eight horses having jugular vein experimental thrombosis induction, four horses attained complete (100%) jugular vein thrombosis, and four attained partial to near-complete venous occlusion of approximately 75, 80, 90, 95% of the jugular vein. One to nine injection periods, of varying vessel occlusion duration, were required to obtain from 75 to 100% vessel occlusion in the eight study horses.

5) The degree of jugular thrombosis remained very consistent in all eight horses over the 30 day experimental study period. Little to no progression or reduction of vessel thrombosis occurred in most horses (7 of 8), and in one horse slight improvement was noted from 75 to 60% over the 30 day study time.

6) No to minimal complications were noted following direct injection of 10-20% ferric chloride into the jugular vein of horses. One horse had an acute, short-lived respiratory distress response following rapid venous injection of ferric chloride, likely due to the high osmolality of the ferric chloride solution. Subsequent slow injections caused no reactions. Another horse had a consistent systemic reaction following each ferric chloride injection, characterized by agitation and muscle fasciculations. The clinical signs appeared similar to a systemic endotoxic response, although a type of anaphylactic reaction could not be ruled out. These reactions responded quickly to systemic intravenous administration of flunixin meglumine.

7) Because the investigators did not have prior experience with the Angio-Vac Cannula system, and Part 2 of the project depended heavily on application of this device in horses, a preliminary pilot project was included in Part 1 of our study to determine the feasibility of using this human vascular cannula in horses. Two of the horses that had previous experimental jugular thrombosis, 75 and 90% vessel occlusion, were chosen for this pilot study. The duration of thrombosis was chronic at approximately 8-10 weeks following experimental vessel thrombosis. The Angio-Vac cannula system was successfully and easily applied in these two experimental horses, although only limited removal of the thrombus was possible at this chronic stage because of the extensive fibrosis of the thrombus within the vessel. This device is recommended and utilized most commonly for removal of acute (<4 days) and sub-acute (4 to 14 days) thrombi in humans, and we hypothesize similar results with younger thrombi in horses.

The $10,000 funding amount for Part 1 of the project was largely divided among equine board fees, hospital charges, pilot testing expenses, and laboratory supplies. The proposed budget for Part 2 is higher than previously estimated. The current budget includes the additional two horses added to the second phase of the study. In addition, the Maquet centrifugal pump (donated for the previously completed Part 1 pilot work in two horses) will be required as a supply item this year as the company will not provide additional product donation. The authors have begun manuscript preparation to describe our findings for Part 1 of this study, experimental jugular vein thrombosis in horses via combined ferric chloride induce endothelial damage and venous stasis. Part 2 of this study, applying the Angio-Vac cannula system to acute and subacute experimental equine jugular thrombosis, is designed and ready for implementation pending further project funding through the Zweig Equine Research Foundation.
**Harry M. Zweig Memorial Fund for Equine Research**

**2014 Final Report**

<table>
<thead>
<tr>
<th>P.I.</th>
<th>Dr. Tracy Stokol</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Title:</strong></td>
<td>The Role of Platelets in the Pathogenesis of Equid Herpes Virus Type-1 Infection</td>
</tr>
<tr>
<td><strong>Project Period:</strong></td>
<td>1/1/12-12/31/14</td>
</tr>
<tr>
<td><strong>Reporting Period</strong></td>
<td>1/1/14-12/31/14</td>
</tr>
</tbody>
</table>

Dr. Stokol was awarded an additional no cost extension through December 31, 2014. A final report is provided.
PROJECT TITLE: The Role of Platelets in the Pathogenesis of Equid herpes virus Type-1 Infection

PRINCIPAL INVESTIGATOR(S): Dr. Tracy Stokol

The aims have not been modified. We have requested and received a no-cost extension to finish Aim 1 and Aim 3.

Describe the studies directed toward specific aims during the current budget year and the positive and negative results obtained. If applicable, address any changes to the innovative potential of the project. If technical problems were encountered in carrying out this project, describe how your approach was modified.

Aim 1: Direct activation of equine platelets by EHV-1: Here we proposed to test whether EHV-1 bound to equine platelets, causing them to become activated, as shown by flow cytometric analysis for P selectin expression (indicating degranulation of platelet α granules) and release of phosphatidylserine (PS)-expressing platelet-derived microparticles (indicating microvesiculation of platelet surfaces).

We have finished this Aim and found that EHV-1 did activate platelets, causing upregulation of P-selectin and microvesiculation with shedding of phosphatidylserine-expressing microparticles. We found that P-selectin expression was mediated through generation of thrombin, likely triggered by tissue factor expressed in the virus envelope, although microvesiculation as partly thrombin- and tissue factor-independent. The study was recently published in PLoS ONE (see attached manuscript).

Aim 2: Indirect activation of equine platelets by EHV-1-infected monocytes: Here we proposed to determine if EHV-1-infected monocytes activate platelets.

We did not work on this Aim because we could not neutralize virus on monocyte surfaces and focused our efforts on Aims 1 and 3.

Aim 3: Indirect activation of equine platelets by EHV-1-infected endothelial cells: Here we proposed to determine if platelets adhere to EHV-1-infected endothelial cells in a microfluidic device.

We have completed a study showing that EHV-1-infected equine carotid endothelial cells upregulate tissue factor and P selectin in response to virus infection. We also found that infected endothelial cells can recruit platelets that have not been exposed to virus, under static and fluidic conditions. Using a novel microfluidic device, we have found that platelets bind to areas of P selectin expression on EHV-1-infected endothelial cells and that rolling and adhesive interactions of platelets with endothelial cells can be blocked by a P selectin inhibitor, fucoidin. The manuscript related to these findings is under preparation.

We are excited to discover that EHV-1 activates platelets and EHV-1-infected endothelial cells become procoagulant, by recruiting platelets through upregulation of P selectin expression. This finding is of tremendous relevance to EHV-1 because platelets are crucial role in thrombosis, one of the causes of abortion and myeloencephalopathy due to EHV-1.
This means, that through inhibiting platelets, we potentially can stop abortion and EHM outbreaks. Indeed, we obtained funding from the Grayson Jockey Club to perform an in vivo double-blinded randomized cross-over treatment study in horses to determine if currently available platelet inhibitors (clopidogrel, aspirin, theophylline and pentoxyfiline) can inhibit EHV-1-induced platelet activation in our in vitro flow cytometric assays (P selectin and microvesiculation). Unfortunately, we found that these drugs were not efficacious in this respect, likely because they do not inhibit thrombin generation, which is a key component of EHV-1-induced platelet activation. In a newly funded Zweig proposal, we are testing standard (low molecular weight and unfractionated heparin) and a new anti-thrombin inhibitor for their ability to block EHV-1-induced platelet activation in vitro using our established assays.
### Harry M. Zweig Memorial Fund for Equine Research

#### 2014 Annual

<table>
<thead>
<tr>
<th>P.I.</th>
<th>Dr. Bettina Wagner</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Title:</strong></td>
<td>Innate Immune Mechanisms and T-Cell Responses to Equine Herpesvirus Type 1 in Latently Infected and Naïve Horses</td>
</tr>
<tr>
<td><strong>Project Period:</strong></td>
<td>1/1/13-12/31/14</td>
</tr>
<tr>
<td><strong>Reporting Period</strong></td>
<td>1/1/14-12/31/14</td>
</tr>
</tbody>
</table>
PROJECT TITLE: Innate Immune Mechanisms and T-Cell Responses to Equine herpes virus Type 1 in Latently Infected and Naïve Horses

PRINCIPAL INVESTIGATOR(S): Dr. Bettina Wagner & Dr. Gillian Perkins

Background
Aim 1 was to optimize our novel neonatal vaccine for induction of immunity against EHV-1 in neonates. We proposed to vaccinate neonates at birth (as previously) and add an additional booster vaccination (new) to further increase immunity and protection against EHV-1. The group size for this experiment was increased to n=7 to (i) confirm and optimize the initial findings and (ii) allow for more statistical power for differences in clinical signs. Aim 1 was designed to provide confirmation for the proof-of-principle from our first vaccine trial in 2012 and to improve the vaccination procedure based on the initial results.

For Aim 1, foals were born and 7 neonates were vaccinated in 2013. Seven foals are maintained as non-vaccinated controls. The vaccinated foals received purified IgE at birth followed by two injections of recombinant EHV-1 antigen that was targeted to the IgE by specific linker molecules. Our working hypothesis is that the EHV-1 antigen crosslinks the IgE on neonatal basophils which in response secrete interleukin 4 (IL-4). IL-4 then acts as B-cell stimulatory factor to induce neonatal B-cell memory responses.

After weaning in January 2014, all foals were challenged with 107 Pfu EHV-1 (strain NY03). Protection from infection and disease and EHV-1 specific immunity were evaluated after experimental infection. Both groups of foals developed a fever and mild signs of respiratory disease. Differences in clinical signs or viral shedding were not observed between groups. However, EHV-1-specific antibody responses in vaccinated foals were significantly higher and started earlier after infection compared to non-vaccinated foals. This result was comparable to our previous neonatal vaccination and challenge study in the project preceding this application. It suggests that the neonatal vaccine induces EHV-1 specific B-cell memory early in life. However, this response did not lead to protection from disease when the foals were infected with EHV-1 several months later. The increased IgE and antigen amounts and an additional boost with EHV-1 antigen compared to the first vaccine trail in 2012 did not improve the clinical outcome.

In Aim 2, was to test how the novel vaccine influences development of memory B-cells in foals after vaccination and EHV-1 infection. B-cell immunity in horses has mainly been investigated at the level of antibody production. The characterization of equine B-cells is still very superficial and studies on memory B-cells do not yet exist. We have identified memory cell markers that cross-react with horse cells which will be used to determine EHV-1-specific B-cell memory in foals after vaccination and experimental...
EHV-1 infection. We will use frozen peripheral blood mononuclear cells from foals that participated in the first vaccination experiment (2012) and the vaccine trial described in this project (2013) to identify and better characterize B-cell memory responses after neonatal vaccination and EHV-1 infection. We will analyze EHV-1-specific memory B-cells by surface staining with IgM (naïve cells) and IgG (memory cells), evaluate cross-reactive memory cell markers and induction of B-cell proliferation. We will measure these parameters by flow cytometry. For Aim 2, flow cytometric measurements of neonatal B-cell memory responses were performed during the infection study in January 2014. These data were fully analyzed and revealed IgM+ and IgG1+ positive B-cells as the main EHV-1 specific populations early after infection. Aim 2 is currently ongoing (see D).

We repeatedly induced significantly increased antibody responses with our neonatal EHV-1 vaccine. The neonatal vaccine is easy to apply. The neonatal vaccine still needs improvement with respect to protection from disease. However, the alternative mechanism of inducing antibody-production to EHV-1 by an IgE-mediated pathway in neonates is novel. A provisional patent application was submitted in fall 2013. Further analysis of foal B-memory cells as outlined in Aim 2 will provide new insights into neonatal and young foal B-cell activation and development. In addition, the following presentations and articles resulted from this project (publications included in the Publications section in the earlier part of this report).
### Harry M. Zweig Memorial Fund for Equine Research

#### 2014 Progress Report

<table>
<thead>
<tr>
<th>P.I.:</th>
<th>Dr. Bettina Wagner</th>
</tr>
</thead>
<tbody>
<tr>
<td>Title:</td>
<td>A Novel Strategy to Boost Antibody Production to EHV-1 in Neonates</td>
</tr>
<tr>
<td>Project Period:</td>
<td>1/1/14-12/31/14</td>
</tr>
<tr>
<td>Reporting Period</td>
<td>1/1/14-12/31/14</td>
</tr>
</tbody>
</table>
Aim 1 was to optimize our novel neonatal vaccine for induction of immunity against EHV-1 in neonates. We proposed to vaccinate neonates at birth (as previously) and add an additional booster vaccination (new) to further increase immunity and protection against EHV-1. The group size for this experiment was increased to n=7 to (i) confirm and optimize the initial findings and (ii) allow for more statistical power for differences in clinical signs. Aim 1 was designed to provide confirmation for the proof-of-principle from our first vaccine trial in 2012 and to improve the vaccination procedure based on the initial results.

For Aim 1, foals were born and 7 neonates were vaccinated in 2013. Seven foals are maintained as nonvaccinated controls. The vaccinated foals received purified IgE at birth followed by two injections of recombinant EHV-1 antigen that was targeted to the IgE by specific linker molecules. Our working hypothesis is that the EHV-1 antigen crosslinks the IgE on neonatal basophils which in response secrete interleukin 4 (IL-4). IL-4 then acts as B-cell stimulatory factor to induce neonatal B-cell memory responses. After weaning in January 2014, all foals were challenged with 10^7 Pfu EHV-1 (strain NY03). Protection from infection and disease and EHV-1 specific immunity were evaluated after experimental infection. Both groups of foals developed a fever and mild signs of respiratory disease. Differences in clinical signs or viral shedding were not observed between groups. However, EHV-1-specific antibody responses in vaccinated foals were significantly higher and started earlier after infection compared to non-vaccinated foals. This result was comparable to our previous neonatal vaccination and challenge study in the project preceding this application. It suggests that the neonatal vaccine induces EHV-1 specific B-cell memory early in life. However, this response did not lead to protection from disease when the foals were infected with EHV-1 several months later. The increased IgE and antigen amounts and an additional boost with EHV-1 antigen compared to the first vaccine trial in 2012 did not improve the clinical outcome.

In Aim 2, was to test how the novel vaccine influences development of memory B-cells in foals after vaccination and EHV-1 infection. B-cell immunity in horses has mainly been investigated at the level of antibody production. The characterization of equine B-cells is still very superficial and studies on memory B-cells do not yet exist. We have identified memory cell markers that cross-react with horse cells which will be used to determine EHV-1-specific B-cell memory in foals after vaccination and experimental EHV-1 infection.

We will use frozen peripheral blood mononuclear cells from foals that participated in the first vaccination experiment (2012) and the vaccine trial described in this project (2013) to identify and better characterize B-cell memory responses after neonatal vaccination and EHV-1 infection. We will analyze EHV-1-specific memory B-cells by surface staining with IgM (naïve cells) and IgG (memory cells), evaluate cross-reactive memory cell markers and induction of B-cell proliferation. We will measure these parameters by flow cytometry.
For Aim 2, flow cytometric measurements of neonatal B-cell memory responses were performed during the infection study in January 2014. These data were fully analyzed and revealed IgM+ and IgG1+ positive B-cells as the main EHV-1 specific populations early after infection. Aim 2 is currently ongoing (see D).

We repeatedly induced significantly increased antibody responses with our neonatal EHV-1 vaccine. The neonatal vaccine is easy to apply. The neonatal vaccine still needs improvement with respect to protection from disease. However, the alternative mechanism of inducing antibody-production to EHV-1 by an IgE-mediated pathway in neonates is novel. A provisional patent application was submitted in fall 2013. Further analysis of foal B-memory cells as outlined in Aim 2 will provide new insights into neonatal and young foal B-cell activation and development. In addition, the following presentations and articles resulted from this project: Invention disclosure - Provisional patent application No. 61/903,619 (US); submitted 11/13/2013; “Stimulation of Neonatal Immunity”. Inventors: B. Wagner, G. Perkins; Applicant: College of Veterinary Medicine, Cornell University, Ithaca, NY (2013).

Oral presentations

Publications

These two publications are not directly related to the proposed EHV-1 project. However, they represent two smaller concise studies that have been performed using the dams of the foals involved in this project and which were partially funded by Zweig funds (acknowledged in both articles).

**Plans**

Summarize plans to address the Specific Aims during the next year of support. Include any important modifications to the original plans.

Aim 1 has been performed, was partially analyzed and the evaluation of all experimental results is ongoing. Publication of data on the neonatal EHV-1 vaccine project has been initially delayed because of the invention disclosure. However, we have started to present some of the data this year and will publish the first article on this approach by the end of next year.

Aim 2 is ongoing. In year 2, we will continue to analyze the different memory B-cell populations in both foal groups at various times after vaccination and experimental infection. As outlined in the original Aim 2, we will use different B-cell markers and cells from the foals that have been stored in liquid nitrogen for this purpose.
APPENDIX B

SUMMARY OF 2014 EXPENDITURES

2014 Research Awards $509,051
2015 Public Relations and Administrative Budget $27,580
2014 Incentive Awards $6,500

Total Expenditures: $543,131
APPENDIX C

RESEARCH PRESENTATIONS
November 12, 2014
Cornell Ruffian Equine Specialists
A Cornell University Affiliate Center for
Equine Sports Medicine & Critical Care
111 Plainfield Ave. Elmont, NY 11003
Novel Strategy to Boost Antibody Production against Equine Herpesvirus-1 in Neonatal Foals

Dr. Gillian Perkins -- Senior Lecturer of Large Animal Medicine
Director of Biosecurity, Cornell University Hospital for Animals
http://www.vet.cornell.edu/faculty/perkins/

Jugular Thrombosis in Horses: A Novel Treatment Approach

Dr. Rolfe Radcliffe -- DVM, Diplomate ACVS and ACVECC
Lecturer, Large Animal Surgery and Emergency Critical Care, Cornell University Hospital for Animals
http://www.vet.cornell.edu/faculty/Radcliffe/

Gene Therapy to Treat Equine Joint Disease

Dr. Kyla Ortved -- Clinical Assistant Professor; Equine Surgeon & Emergency Clinician, Cornell ruffian Equine Specialists
http://ruffian.cornell.edu/Ortved/

Investigating the Role of Pili in the Pathogenesis of Streptococcus equi

Dr. Helene Marquis -- Associate Professor of Microbiology, Cornell Veterinary Medicine
http://www.vet.cornell.edu/microbiology/faculty/Marquis

Putting the Horse First

Dr. Scott Palmer – VMD, NYS Equine Medical Director, Adjunct Professor, Cornell Veterinary Medicine
http://www.vet.cornell.edu/news/ScottPalmer.cfm

Ruffian Overview

Dr. Samuel Hurcombe -- Associate Clinical Professor, Cornell Ruffian Equine Specialists
http://ruffian.cornell.edu/hurcombe/

-- Tour of Ruffian & Reception --
http://www.vet.cornell.edu/zweig
Faculty and staff from Cornell University’s College of Veterinary Medicine, Ithaca, New York, and Cornell Ruffian Equine Specialists, Elmont, New York, presented an array of equine-related research projects and lectures on November 12, 2014 at the Ruffian facility. Members of the Harry M. Zweig Memorial Fund for Equine Research and professional staff attended the events learning of the success of currently funded projects, and hearing all about upcoming research projects.

Speakers included Gillian Perkins – Senior Lecturer of Large Animal Medicine, Director of Biosecurity, Cornell University Hospital for Animals, Rolfe Radcliffe, DVM, Diplomate ACVS and ACVECC, Lecturer, Large Animal Surgery and Emergency Critical Care, Cornell Hospital for Animals, Kyla Ortved, Clinical Assistant Professor, Equine Surgeon & Emergency Clinician Cornell Ruffian Equine Specialists, Helene Marquis, Associate Professor of Microbiology, Cornell Veterinary Medicine, Scott Palmer, VMD, NYS Equine Medical Director, Adjunct Professor, Cornell Veterinary Medicine, and Samuel Hurcombe, Associate Clinical Professor, Cornell Ruffian Equine Specialists, with their respective lecturers on, “Novel Strategy to Boost Antibody Production against Equine Herpesivirus-1 in Neonatal Foals,” Jugular Thrombosis in Horses: “A Novel Treatment Approach,” “Gene Therapy to Treat Equine Joint Disease,” Investigating the Role of Pili in the Pathogenesis of Streptococcus equi,” “Putting the Horse First,” and Dr. Hurcombe gave an overview of the Ruffian Equine Specialists facility, located opposite the backstretch of historic Belmont Park. Ruffian Equine Specialists extends the reach of the Cornell Equine Hospital, where internationally renowned specialists inspire and capitalize on the synergy between the science and art of medicine. Cornell equine specialists leverage their knowledge, experience, and professional partnerships - including those with Cornell College of Veterinary Medicine colleagues who offer depth and breadth across the spectrum of specialties to provide excellent specialty care in state-of-the-art facilities that promote the health and well-being of horses.

At the annual meeting on November 13, 2015 at Ruffian, long-time member and leader of the Harry M. Zweig Memorial Fund for Equine Research, Mrs. Anna Zweig, announced her plans to retire from the Zweig Committee effective immediately. In her farewell speech, she said her late husband, Harry Zweig, would have been very pleased at the success and impact of the Zweig Fund, and thanked past and present committee members for their dedication and service. It was time for her to step down, she said, to make way for someone more involved in the day-to-day activities of the fund, expressing her great pleasure that Dr. Ann Dwyer, New York Practitioner agreed to serve on the Zweig Committee effective 2015. Dr. Dwyer is a member of AAEP, AVMA, NYSVMS and IEOC, and she served as President of the American Association of Equine Practitioners in 2013. Michael I. Kotlikoff, VMD, PhD, Austin O. Hooey Dean of Veterinary Medicine at Cornell thanked Mrs. Zweig for her many years of dedication, support, and leadership, expressing his sadness at her departure from the committee, and noting that the Zweig name would still be represented on the Committee through her son, Brian Zweig, a member since 2007.
The Harry M. Zweig Memorial Fund for Equine Research honors the late Dr. Harry Zweig, a distinguished veterinarian, and his numerous contributions to the state’s equine industry. In 1979, by amendment to the pari-mutuel racing and wagering law, the New York State legislature created the Harry M. Zweig Memorial Fund for Equine Research to promote equine research at the Cornell University College of Veterinary Medicine. The Harry M. Zweig Committee was established for the purpose of administering the fund and is composed of individuals in specified state agencies and equine industry positions and others who represent equine breeders, owners, trainers, and veterinarians. The Fund contributes a percentage of its revenue to support a variety of equine-related research. The Fund is proud to support the Harry M. Zweig Memorial Fund for Equine Research. This first-rate research helps to provide protection and preventative planning for the equine industry, which in turn helps to ensure a healthy and positive future for the horse racing industry.

The committee administering the fund always includes the chairman of the New York State Racing and Wagering Board or his designee, the dean of the College of Veterinary Medicine at Cornell or his designee, a member or the executive director of the Agriculture and New York State Horse Breeding Development Fund, a member or the executive director of the New York State Thoroughbred Breeding and Development Fund, and at least five New York State breeders, owners, trainers, or veterinarians in equine practice. Dean Michael Kotlikoff currently serves on the committee, representing the College and its many researchers who have received the Fund’s support for research projects advancing equine health and athleticism.
Thanks, Anna, for your support, dedication and leadership for over 30 years!!

See you at the Harry M. Zweig Memorial Trot – Sunday, July, 26 2015 –
Post Time 1:15pm at Vernon Downs!
http://www.vernondowns.com/racing/horsemen.php
APPENDIX D

AWARDS FOR 2015
APPENDIX D
2015 Harry M. Zweig Memorial Fund for Equine Research Awards

<table>
<thead>
<tr>
<th>CONTINUATION</th>
<th>ANNUAL AWARD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr. Nixon</td>
<td>Evaluation of Lubricant as a New Biotherapeutic for Equine Joint Disease (Year 2)</td>
</tr>
<tr>
<td>Dr. Wagner</td>
<td>A Novel Strategy to Boost Antibody Production to EHV-1 in Neonates (Year 2)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>NEW/Renewal</th>
<th>ANNUAL AWARD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr. Cheetham</td>
<td>Regenerative Approach to Recurrent Laryngeal Neuropathy (1 Year award)</td>
</tr>
<tr>
<td>Dr. Fortier</td>
<td>Subtle Meniscal Injury as an Initiating Event in the Development of Subchondral Bone Cysts (1 Year award)</td>
</tr>
<tr>
<td>Dr. Gilbert</td>
<td>Controlled Postponement of Ovulation in Mares (2 Year award)</td>
</tr>
<tr>
<td>Dr. Marquis</td>
<td>Bacterial pilus as Vaccine Target for Strangles (1 Year award)</td>
</tr>
<tr>
<td>Dr. Nixon</td>
<td>Enhanced Breakdown Screening in Thoroughbred Racehorses through Multimodal Imaging and Serum Biomarker Combinations (2 Year award)</td>
</tr>
<tr>
<td>Dr. Ortved</td>
<td>Harnessing the Immunomodulatory Properties of Interleukin-10 through a Gene Therapy Approach to Prevent Equine Osteoarthritis (1 Year award)</td>
</tr>
<tr>
<td>Dr. Palmer</td>
<td>Epidemiology Acute Breakdown Study (1 Year award)</td>
</tr>
<tr>
<td>Dr. Radcliffe</td>
<td>En Bloc Removal of Intravascular Thrombi via an Extracorporeal Bypass Circuit in Experimentally Induced Jugular Thrombosis in Horses (1 Year award)</td>
</tr>
<tr>
<td>Dr. Stokol</td>
<td>Platelets are a Trojan Horse that Deliver Equine Herpes Virus to Endothelial Cells (2 Year award)</td>
</tr>
</tbody>
</table>

Sub-Total: $171,761

TOTAL: $595,446
APPENDIX E

ZWEIG NEWS CAPSULES
Cornell opens equine hospital close to Belmont

Cornell’s College of Veterinary Medicine opened Cornell Ruffian Equine Specialists, a referral and emergency care hospital, near the Belmont Racetrack backstretch on Long Island in Elmont, N.Y. on March 31, 2014.

The College has signed a lease-buy agreement with Racebrook Capital Advisors LLC for the former Ruffian Equine Medical Center. Consistent with Zweig’s longstanding interest in equine health, the new hospital will provide elective equine specialty services to horses referred by their attending veterinarians. Full emergency and critical care services are also available.

Cornell Ruffian Equine Specialists will partner with referring veterinarians to meet the needs of the New York state racing and surrounding sport horse communities. The 22,000-square-foot facility will provide state-of-the-art surgical, imaging, diagnostic and rehabilitation services to enhance equine health.

Dr. Alan Nixon, a renowned equine orthopedic surgeon and director of the Comparative Orthopedics Laboratory, will serve as the chief medical officer. Drs. Lisa Fortier and Norm Ducharme, pioneers in regenerative and laryngeal procedures, will also offer advanced surgical procedures. The hospital will be staffed by Cornell veterinarians and technicians and will offer a full complement of advanced orthopedic and soft tissue surgery and regenerative therapies, an internal medicine service and a broad array of diagnostic modalities, including advanced imaging such as MRI, CT, nuclear scintigraphy, high-speed treadmill endoscopy, arthroscopy and laboratory services.

“We are looking forward to joining the well-established horse-racing and sport horse communities in the area, adding value to veterinarians, trainers and owners in the region and supporting the critical equine industry in the state of New York,” said Dr. Michael I. Kotlikoff, the Austin O. Hooey Dean of Veterinary Medicine at Cornell. “The hospital will be within walking distance of Belmont Park, recognized as one of the world’s premiere thoroughbred horse-racing facilities, and is easily accessible to the many sport horse enthusiasts located in and around the area. Our goals are to improve the health and safety of the equine athlete and by so doing to strengthen one of the world’s premiere racing programs.”

“This is an exciting initiative for Cornell,” said Nixon. “Through the establishment of Cornell Ruffian Equine Specialists, Cornell will honor Ruffian’s legacy. She established herself as one of the greatest racehorses to set foot on the track and is known as the perfect champion and a courageous filly. The new center will continue the sense of inspiration and achievement surrounding Ruffian, and we are eager to partner with the referring veterinarians to do so. We have multiple goals for our new hospital, all of which are patient-centered, client-responsive and community-minded.”
Equine surgical innovations

A pair of unique surgical procedures performed on animals promises to revolutionize the ways surgeons repair cartilage and meniscus tears. In the first set of procedures, a cross-institutional, interdisciplinary team of surgeons and researchers tried a new method for cartilage repair on horses at the Cornell University Hospital for Animals on August 21.

Another team conducted a meniscus repair procedure on September 3 and 4 involving custom-designed, individualized replacement parts. With information from an MRI scan of the patient’s joint, the researchers used a 3-D printer to assemble an artificial meniscus fitted to the patient’s body.

The surgeons and researchers taking part in these groundbreaking trials include medical staff from Cornell’s College of Veterinary Medicine, including Zweig grant recipient Dr. Lisa Fortier; the Hospital for Special Surgery (HSS), a section of the NewYork-Presbyterian Healthcare System and an affiliate of Weill Cornell Medical College in New York City; and the New York Giants football team.

“The goal is to make these technologies available for both horses and people,” said Lisa Fortier, professor of large animal surgery at Cornell’s College of Veterinary Medicine.

The August 21 procedures involved one-hour surgeries on five horses to practice a new procedure and use a cartilage repair device developed by a biomechanical engineer at HSS who designed an off-the-shelf biocompatible scaffolding that can be surgically inserted into damaged cartilage for repair and to prevent the onset of arthritis. Cartilage defects occur due to overuse or from such traumatic episodes as a crucial ligament tear or rupture, for example.

“We know that any of those types of cartilage defects can lead to arthritis,” which the procedure aims to prevent, Fortier said. Once cartilage is torn, there is currently no viable repair nor does it heal on its own.

“My goal is to be able to use these procedures in my own equine patients,” said Fortier.
Dr. Bettina Wagner has been appointed Associate Dean for Research and Graduate Education at the Cornell University College of Veterinary Medicine. Her five year term began September 1, 2014. Dr. Joel Baines, who previously held the position, became the Dean of Louisiana State University’s School of Veterinary Medicine effective September 1, 2014.

Wagner’s research has focused on equine immunology, particularly immune responses and protective mechanisms in neonates and young foals. For this work she was honored with a two-year term as the Harry M. Zweig Assistant Professor in Equine Health in 2009.

“Bettina is an outstanding scientist who has made major contributions to the detection of animal diseases and brings enormous credibility to this important leadership position,” said Dr. Michael Kotlikoff, the Austin O. Hooey Dean of Veterinary Medicine.

As Associate Dean, Wagner will be responsible for the strategic leadership and advancement of research and graduate education at the College. She will oversee research administration across the College, forging collaborative partnerships between researchers and other stakeholders across the College.

Managing the College Research Office, which facilitates grant applications, she will forge opportunities for faculty collaborations; implement research initiatives; serve as Chair of the College Research Affairs Committee. In addition she will provide administrative oversight for internal grant programs, including the Zweig Memorial Fund Program; serve as Director of the Veterinary Investigator Program and as Associate Director of the Cornell University Agriculture Experiment Station, and provide strategic leadership for the College’s research programs and summer research programs.

With support from the Harry M. Zweig Memorial Fund for Equine Research for over 30 years, the College of Veterinary Medicine has been able to conduct cutting edge research benefiting the equine species, which helps provide protection and preventative planning for the equine industry, thus helping to ensure a healthy and positive future for the horse racing industry. By legislation, the Zweig Fund receives a percentage of revenue to support research benefitting equine health. Cornell has developed equine research projects in the following areas supported, in part, by the Zweig Fund: reproduction, orthopedics, genetics, cardio-respiratory functions, nutrition, and infectious diseases.

In addition, the Zweig Fund has been instrumental in supporting the careers of young equine researchers through the Harry M. Zweig Assistant Professor in Equine Health program and the Zweig Equine Clinical Fellowship program.

To mark the 35th anniversary of the Fund, we wish to demonstrate the College’s appreciation and to share its accomplishments by hosting a series of research presentations for the Zweig Committee. The presentations will be in support of the Harry M. Zweig Memorial Fund, and are intended to further promote the efforts of the Fund and to demonstrate a greater awareness of equine health and research.

The presentations are scheduled for mid-November at the Cornell Ruffian Equine Specialists, a Cornell University Affiliate center for equine sports medicine and critical care located at 111, Plainfield Avenue, Elmont, NY 11003. Learn more at: ruffian.cornell.edu/
The Harry M. Zweig Memorial Fund for equine Research honors the late Dr. Harry M. Zweig, a distinguished veterinarian, and his numerous contributions to the state’s equine industry. In 1979, by amendment to the pari-mutuel revenue laws, the New York State legislature created the fund to promote equine research at the College of Veterinary Medicine, Cornell University. The Harry M. Zweig Committee is established for the purpose of administering the fund and is composed of individuals in specified state agencies and equine industry positions and others who represent equine breeders, owners, trainers, and veterinarians.

Let’s talk ticks

Cornell University’s Animal Health Diagnostic Center (ahdc.vet.cornell.edu) answers frequently asked tick questions:

What should I do if I find a tick attached to me or my horse?
Don’t panic. Remove the tick with a good sharp set of tweezers and protective gloves. There are also various products for tick removal on the market of variable efficacy. Do not attempt to burn or suffocate the tick as this causes the tick to release additional, potentially infectious, saliva into the wound. Observe the feeding site for signs of infection. Keep the tick in an escape proof container pending further testing.

What are the dangers of ticks?
Ticks are vectors for a number of diseases. After they attach to the horse, they can begin transmitting infectious diseases, such as Anaplasmosis. For other pathogens like Borrelia burgdorferi, the causing agent of Lyme disease, it takes 18-24 hours before the bacteria are transmitted. Not all ticks carry pathogens.

What is Lyme disease and how common is it?
Lyme disease is the most common vector-borne disease in the United States. It is transmitted by ticks. The disease affects humans, dog, horses and possibly other animals. Lyme disease is caused by infection with bacteria in the genus Borrelia. Only some horses that get bitten by infected ticks will also develop Lyme disease.

How do I know if a tick is infected with Lyme disease?
The percentages of pathogen-infected ticks vary from region to region. Lyme disease is endemic in the northeastern part of the US and occurs less frequently in other regions. Only a proportion of ticks are infected with Anaplasma or Borrelia. The Animal Health Diagnostic Center at Cornell University offers tick identification and PCR to identify if a tick was infected with Anaplasma or Borrelia. If the tick was infected with Borrelia, a follow up Lyme Multiplex test on the horse’s blood should be performed three to four weeks after the tick was removed.

Mark D. Gearan is the president of Hobart and William Smith Colleges in Geneva, N.Y. He previously served in many public leadership roles, including Director of the Peace Corps and Assistant to the President, Director of Communications, and Deputy Chief of Staff to President Bill Clinton. He is an appointee of the Bipartisan Policy Center’s Commission on Political Reform. Gearan earned his B.A. in government at Harvard University and his law degree at Georgetown University.

CORNELL UNIVERSITY COLLEGE OF VETERINARY MEDICINE 2015 HARRY M. ZWEIG MEMORIAL FUND COMMITTEE

Jean Brown
Sr. Vice President, Operations
Blue Chip Farms, Inc.
Wallkill, N.Y.

Gabriel Cook, DVM
New England Equine Practice
Patterson, N.Y.

Janet Durso, DVM
Middletown, N.Y.

Mark D. Gearan
Chairman
New York State Gaming Commission
Schenectady, N.Y.

Paul Kelley
Kelley Racing Stable, LLC
Gansevoort, N.Y.

Michael I. Kotlikoff, VMD, PhD
Austin O. Hooye Dean of Veterinary Medicine
Cornell University College of Veterinary Medicine, Ithaca, N.Y.

Paul C. Mountan, DVM
Rhinebeck, N.Y.

Robert M. Tugel, DVM
Avon, N.Y.

Patricia Wehle
Fairport, N.Y.

Robert Williams
Executive Director
New York State Gaming Commission
Schenectady, N.Y.

William Wilmot, DVM
NYS Thoroughbred Breeding & Development Fund Corp. Saratoga Springs, N.Y.

Anna Zweig
Middlebrook Farms
Nassau, N.Y.

Brian Zweig
Rensselaer, N.Y.
Winner’s Circle
“Shake it Cerry” (top) and “Father Patrick” (bottom), winners of this year’s Zweig Trot held at Vernon

Prefer to receive this newsletter electronically? Please email lam78@cornell.edu with the subject “Subscribe Zweig newsletter.” We will add your email address to a list and remove your name from the print mailing list. You can read the searchable electronic newsletter online or download at your convenience at http://bit.ly/ZweigNews.
2014 Research Awards

New
$60,960 to Dr. Dorothy Ainsworth for “Fine Mapping of Candidate Genes Contributing to Equine Left Recurrent Laryngeal Neuropathy (RLN) in Thoroughbred Horses – Phase II”

$49,350 to Dr. Robert Gilbert “Effect of Early Pregnancy on Function of the Equine Corpus Luteum”

$173,259 to Dr. Alan Nixon for “Evaluation of Lubricin as a New Biotherapeutic for Equine Joint Disease”

Continued
$50,000 to Dr. Douglas Antczak with Rebecca Tallmadge and Nikolaus Osterrieder for “T-cell Mediated Immunity and Vaccine Development in Horses”

$67,000 to Dr. Thomas Divers with Dr. Bud Tennant for “Etiology and Prevention of Equine Serum Hepatitis (Theiler’s Disease)”

$88,499 to Dr. Bettina Wagner with Gillian Perkins for “Innate Immune Mechanisms and T-cell Responses to Equine Herpesvirus Type 1 in Latently Infected and Naïve Horses”

Revised / Renewed

$52,696 to Dr. Lisa Fortier for “Cellular Biomarkers of Early Cartilage Injury Measured in vivo with Multiphoton Imaging.”

$162,786 to Dr. Bettina Wagner for “A Novel Strategy to Boost Antibody Production to EHV-1 in Neonates”

$10,000 to Dr. Rolfe Radcliffe for “EnBloc Removal of Intravascular Thrombi via an Extracorporeal Bypass Circuit in Experimentally Induced Jugular Thrombosis in Horses”
A unique feature of equine pregnancies

In horses and their close relatives, unusual cells of the placenta invade the mother’s womb during early pregnancy. Called endometrial cups, they behave much like cells from metastatic tumors, leaving the placenta and migrating into the uterus, where they secrete the pregnancy hormone, equine Chorionic Gonadotrophin (eCG). Although endometrial cup cells are unique to the horse family, similar invasive cells have been described in human placentas. In both humans and horses these invading placental cells interact with the mother’s immune system and are thereby thought to contribute to maternal immunological tolerance of the fetus.

Dr. Doug Antczak was recently invited to commemorate the 100th anniversary of the discovery of the endometrial cups in the inaugural volume of the Annual Review of Animal Biosciences. The paper describes the progression of discoveries in reproduction, evolution, and immunology that followed, as well as future questions that remain to be addressed. It documents the milestones in the study of endometrial cups, but it is also a testament to the success of the Zweig Fund as a program to jumpstart research relevant to equine health. In 1979 one of the first Zweig Fund grants was awarded to Antczak, who had just begun his faculty appointment at Cornell. That project, on maternal immune recognition of pregnancy, began a 35 year collaboration and friendship between Antczak and Dr. W. R. Allen.

In the following years Antczak and Allen and their students and staff discovered how placental cells, particularly endometrial cups, control expression of histocompatibility genes to avoid destruction by the mother’s immune system. They learned how the mother’s immune system is regulated during pregnancy to prevent deleterious anti-fetal immune reactions. Finally, they developed and characterized models of pregnancy failure using embryo transfer between horses and donkeys and the use of sterile hybrid mules as embryo recipients. Taken together, these studies have advanced our understanding of how mother, fetus, and placenta communicate and compromise during pregnancy to bring about the miracle of birth.

This fruitful collaboration is an excellent example of how the Zweig Fund has enabled equine scientists and clinicians at Cornell to build strong long-lasting research programs. Such programs have attracted additional funding from national agencies and foundations, produced new information and clinical applications, and provided training for a generation of students who are now pursuing successful independent careers.
Locating Lyme disease

Roming through summer fields seems like a harmless pleasure for horses. But just one bite from the wrong tick can rob an animal of that pastime. The bacteria Borrelia burgdorferi live in certain species of ticks, and can infect animals the ticks bite with Lyme disease. Lyme disease can cause a slew of debilitating symptoms from arthritis to outright lameness, cardiac complications, kidney disease, and neurological symptoms from chronic pain and weakness to paralysis. It’s important to diagnose the disease early because it becomes progressively harder to treat as the bacteria hide in the joints and organs of their hosts.

“The bacteria that cause Lyme disease are particularly difficult to detect,” explained Dr. Bettina Wagner, associate professor in the Department Of Population Medicine and Diagnostic Sciences and director of serology at the Animal Health Diagnostic Center (AHDC) at Cornell. “After infection they tend to hide where they can’t be detected. They bury in the joints of dogs, causing arthritis or lameness, or in severe cases kidney disease, the so-called ‘Lyme nephritis’. In humans and horses they can also enter the central nervous system, causing pain, paralysis, or behavioral alterations. By the time such clinical signs appear, the bacteria are not in circulation anymore and cannot be detected by tests that target the pathogen directly.”

Infection with the bacteria causes the immune system to produce antibodies, protective proteins in the blood, specially tailored to identify, bind and fight specific pathogens such as harmful bacteria. Diagnosticians can test blood samples to see whether an animal made antibodies in response to B. burgdorferi bacteria. If the antibodies are detectable, the animal is likely infected.

“The Lyme Multiplex assay has been offered through the AHDC at Cornell since 2011,” said Wagner, “The new test exceeds its predecessor in accuracy, specificity, and analytical sensitivity. It is fully quantitative which is important to make treatment decisions and to follow-up on treatment success.”

The Lyme Multiplex assay for horses and dogs was developed by Wagner and her colleagues at Cornell. It detects antibodies to three different antigens of B. burgdorferi simultaneously in one test. Multiplex technology has been around for the last decade, but the Animal Health Diagnostic Center (AHDC) is the first veterinary diagnostic laboratory that used it for Lyme disease testing. Different kinds of antibodies can be found in the body at different stages of infection. The new test can distinguish and measure these differences, giving more information about the disease.

“With the Lyme Multiplex assay, we can not only distinguish between infection and vaccination, but between early and chronic infection stages,” Wagner noted. “That was not possible before the Lyme Multiplex Assay was available. Previously, we were able to say whether an animal was infected, but not when or how far the infection has developed.”

The test helps veterinarians to make advanced decisions about treatment. Antibiotic treatment of Lyme disease is much more effective during the early infection stages. The longer the infection persists, the more difficult it gets to treat or cure. If veterinarians decide to treat an animal for Lyme disease, they usually conduct Lyme Multiplex follow-up testing to see if the treatment was successful.

“We look at the improvement of clinical signs and for a clear decline of antibodies in the blood,” Wagner said. “With the information the Lyme Multiplex assay gives us before and after treatment, we can measure its success and better manage Lyme disease in animals.”
Breaking down blood clots

A clot cutting off blood to the wrong place can spell disaster or death for unborn foals and even adult horses. When the infectious disease equine herpes virus-1 (EHV-1) causes its infamous effects, abortions and adult neurological disease, blood clots are to blame.

Clinical pathologist Dr. Tracy Stokol has been investigating how the virus triggers these clots. Her Zweig-funded work investigating the role of platelets in the pathogenesis of EHV-1 infection has shown that EHV-1 virus particles seem to be binding to platelets, small cells in blood involved in clotting.

When incubated together with platelets at a multiplicity of infection (MOI) of 1 – a ratio of 1 virus particle per platelet – particles of the neuropathogenic strain Ab4 and abortion-inducing strain RacL11 induced platelet activation within 10 minutes. Activation causes the release of P selectin, a protein that platelets use to bind to other cells, such as the cells lining blood vessels. The viral gene product glycoprotein B was also amplified from platelets, suggesting that the virus is binding to them directly.

“We are excited to discover that EHV-1 activates platelets,” said Stokol. “Platelets play a crucial role in thrombosis, a major cause of abortion and neurological symptoms due to EHV-1. If platelets are involved in the pathogenesis of these EHV-1-associated disease syndromes, administration of platelet-inhibiting medications such as Plavix or aspirin may prove useful in the treatment of infected horses.”

Stokol is planning several experiments to address the many remaining questions regarding how EHV-1 activates platelets. These include projects that will determine if virus-mediated platelet activation requires other clotting proteins in blood, if the virus uses known cell receptors, such as MHCII, to bind to and then activate platelets, and if inhibitors, such as Plavix and aspirin, can prevent the virus-induced platelet activation.

She is continuing an innovative technique involving growing equine endothelial cells, which line blood vessels, in a microfluidic device to determine whether virus-activated platelets show increased binding to these cells, which could spur clotting and potentially inflammation.

From left: Drs. Alan Nixon, Michael Kotlikoff, Lisa Fortier, Samuel Hurcombe, Lorin Warnick, Norm Ducharme, and Gabriel Cook.